CLINICAL PRACTICE

Resistance to pyrazinamide and ethambutol compromises MDR/XDR-TB treatment

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The increase in multidrug-resistant tuberculosis (MDR-TB), defined as *Mycobacterium tuberculosis* resistant *in vitro* to at least isoniazid (INH) and rifampicin (RIF), is a global concern. It is estimated that 511 000 MDR-TB cases occur globally each year. The World Health Organization (WHO) consequently released an emergency update on their management guidelines, recommending that treatment of MDR-TB should include at least 4 effective drugs, and that standardised treatment regimens should be based on resistance patterns for each country/region. Most importantly, treatment regimens should not depend on the results of drug susceptibility testing (DST) for ethambutol (EMB) or pyrazinamide (PZA). In response, the South African Department of Health prepared a draft drug-resistant TB treatment policy in which PZA remains one of the 4 effective drugs, while EMB should be replaced with terizidone or cycloserine, if there is resistance to EMB (disregarding inaccurate DST). In South Africa, there is a high frequency of undetected EMB and PZA resistance and their association with MDR-TB. Therefore, we recommend that the WHO guidelines in which 4 other effective drugs are used to treat MDR-TB, be followed more closely. EMB and PZA can be included if they are not counted as one of the 4 effective drugs. However, this does not address the root cause of the amplification of resistance in undiagnosed MDR-TB patients in South Africa, which can only be achieved by the implementation of rapid DST methods in all TB cases before initiating therapy. This protocol would curb the amplification of resistance and the evolution of XDR-TB.

The number of MDR-TB cases has steadily increased despite the widespread implementation of the directly observed treatment short-course (DOTS) and DOTS-plus strategies.[1,2]

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A survey in South Africa estimates that 1.8% of new TB cases and 6.7% of retreatment TB cases are MDR-TB,[1] equating to approximately 14 000 MDR-TB cases each year.[1] The manner in which the WHO DOTS strategy has been implemented in South Africa to control TB might have inadvertently lead to the amplification of resistance in MDR-TB cases.[3-5] Consequently, it is important to review the epidemiology of drug resistance in the country and make informed evidence-based suggestions on improving the current treatment strategy.

The WHO DOTS-plus treatment guidelines for treating MDR-TB focus on the use of various bactericidal or bacteriostatic antituberculosis drugs with a proven efficacy against *M. tuberculosis*. They are classed as either first-line (normally used to treat new and drug-susceptible TB cases) or second-line (normally used to treat MDR-TB or extensively drug-resistant TB (XDR-TB)). EMB and PZA, two first-line drugs, are often used in combination with various second-line drugs to treat MDR/XDR-TB. Their inclusion in the latter treatment regimen was based on the absence of alternative second-line drugs and surveillance data (or lack thereof), which suggests that resistance to EMB and PZA is rare.[6] However, DST for both EMB and PZA is inaccurate.[7,8] The National Health Laboratory Service (NHLS) in Cape Town missed 90% of EMB resistance using the indirect proportion method on Middlebrook solid medium compared with a liquid culture medium.[7] Of the EMB-resistant isolates, 87% were also resistant to INH and RIF. These results were confirmed by DNA sequencing which identified mutations in the *embB* gene which conferred resistance to EMB in all the above isolates.[7,9] In 2008, we showed in a cohort of 228 MDR-TB isolates from the Western Cape that 131 (57.5%) harboured mutations in the *embB* gene, suggesting that they were resistant to EMB (unpublished data). Only 9.4% were phenotypically resistant by routine culture on solid media. In a drug resistance surveillance study in children in the Western Cape from March 2007 through February 2009, 12 out of 24 (50%) with confirmed MDR-TB were phenotypically resistant to EMB, confirming the high rate of EMB resistance in adult MDR-TB cases (HSS, personal communication).

DST for PZA is not routinely performed in South Africa owing to the complexity of the culture conditions (low pH medium is required, which negatively affects the growth and viability of *M. tuberculosis*).[10] To address the largely unknown

frequency of resistance to PZA, a recent study (using the non-radiometric BACTEC mycobacteria growth indicator tube (MGIT) 960 method) showed that 53.5% of drug-resistant isolates (various resistance patterns) from the Western Cape were resistant to PZA. This finding was confirmed by DNA sequencing of the *pncA* gene, which encodes for the mechanism of resistance.[8,11] This study importantly showed a highly significant association between MDR-TB and PZA resistance ($p<0.001$)[8] that was confirmed in isolates collected as part of a national drug-resistance survey where 52.1% of MDR-TB isolates showed additional resistance to PZA.[6]

The association between EMB and PZA resistance and MDR-TB in South Africa (a setting where MDR-TB is primarily transmitted[12]) stems from the manner in which the DOTS strategy has been implemented since implementation in 1996. New TB cases are routinely diagnosed by sputum smear microscopy or culture without DST. In the absence of routine DST, it is assumed that all new TB cases are drug-susceptible until treatment failure or relapse occurs. Therefore, according to the current 2004 South African treatment guidelines (South African National Tuberculosis Control Programme Practical Guidelines 2004), a new TB patient with undiagnosed MDR-TB will be treated with 4 first-line drugs (INH, RIF, PZA and EMB) during the intensive phase of therapy (the first 2 months), which implies that the treatment regimen will contain only 2 effective drugs: EMB and PZA. Since PZA is a poor companion drug to prevent the acquisition of resistance, it is highly probable that resistance to EMB and/or PZA will follow, as is evident by the above data. If patients fail to show sputum conversion after 5 months of treatment, they would be regarded as a treatment failure and be shifted to the category II regimen (i.e. South African current retreatment guidelines) with the addition of streptomycin (SM) in the first 2 months of retreatment. DST will then be requested (which may take months[14]), during which time resistance to any remaining effective drugs may develop (i.e. EMB, PZA and/or SM). Consequently, the MDR-TB epidemic will become largely associated with EMB, PZA and SM resistance. In addition, during the diagnostic delay period, transmission to close contacts may occur, thereby perpetuating the MDR-TB epidemic.

According to the South African National Tuberculosis Control Programme Practical Guidelines 2004, a patient will only be placed on the standardised treatment for MDR-TB after DST results become available. This could be at least 6 - 7 months after initial therapy started. The patient will be placed on the current standardised MDR-TB treatment regimen which includes a fluoroquinolone (usually ofloxacin or ciprofloxacin), amikacin (AM) or kanamycin (KM), ethionamide (for which the strain may be resistant if inhA promoter region mutation is the cause of INH resistance), PZA and EMB (replaced with cycloserine or terizidone if resistant to EMB). Resistance to PZA,[8] EMB[7] and ethionamide[13] is commonly associated with MDR-TB and, if not detected, patients with MDR-TB may only receive 2 effective drugs (a fluoroquinolone and AM or KM) of which AM or KM is not used during the 12 - 18-month continuation phase of therapy. This situation could lead to unintentional monotherapy during the continuation phase, with possible acquisition of resistance to the fluoroquinolones, leading to pre-XDR-TB (one resistance mutation away from XDR-TB).[12] Therefore, many MDR/XDR-TB cases are presently being inadvertently under-treated, which may strongly influence treatment outcome. This scenario may be repeated as treatment regimens are adjusted, leading to the eventual evolution of XDR-TB.[4]

To address the difficulties associated with DST for EMB and PZA, the WHO in 2008 released an emergency update on guidelines for treating drug-resistant TB in which they acknowledged that MDR-TB treatment regimens should not be dependent on the results of DST for EMB or PZA.[15] They recommended that treatment of MDR/XDR-TB should include at least 4 drugs with certain or almost certain effectiveness. Treatment regimens can be individualised or standardised if resistance patterns for the country/region are known. EMB could be included in a regimen provided that it is not counted as one of the effective 4 drugs, and that PZA may be used for the entire treatment if deemed effective (based on DST) but must also not be counted as one of the 4 effective drugs. Ciprofloxacin is no longer recommended for treatment of TB.[15]

New draft policy guidelines (2008) are being formulated by the South African National TB Control Programme based on the WHO recommendations. These guidelines aim to address diagnostic delay as well as treatment of MDR/XDR-TB. They suggest that DST should be done on patients failing to show clinical or bacteriological improvement at 2 months of treatment or at 3 months in cases where the intensive phase was extended (as opposed to 5 months in the old guidelines). In contrast to the WHO, these guidelines recommend that DST for EMB determines the treatment regimen for MDR/XDR-TB. If susceptible, EMB should be included in the regimen as the 5th drug (this disregards the inaccuracy of EMB DST). If resistant, EMB should be replaced with terizidone or cycloserine as the 5th drug. These guidelines also recommend the inclusion of PZA as one of the 4 effective drugs but acknowledge that, in the absence of DST, it should be assumed that all isolates are resistant. Given the strong evidence of high levels of resistance to PZA in South Africa associated with MDR-TB, we suggest that, in the absence of DST for PZA, this drug should not be counted as one of the 4 effective drugs, but may be included in the regimen. In light of the WHO recommendations and the high levels of undetected resistance to EMB and PZA in South Africa, it is essential that the revised guidelines for MDR-TB treatment are implemented in South Africa together with improved routine DST. In the interim, it

should be assumed that all MDR-TB cases are resistant to EMB and PZA and, although treatment with these drugs can be continued, they should not be counted as one of the 4 effective drugs, to prevent the emergence of additional resistance and the possible evolution of XDR-TB.

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